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Biodegradation in the Estuarine-Marine Environments and the Genetically Altered Microbe

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16. ABSTRACT

Historically, some organic and inorganic compounds have been considered serious environmental threats from the standpoint of quantity produced, bioaccumulation, toxicity, or adverse environmental effects. This paper documents selected examples of known introductions of toxic chemicals into marine and estuarine environments and describes how habitat differences affect biodegradation potential. Concerns regarding release of genetically altered organisms into these environments also are discussed.

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Al W. Bourquin

U.S. Environmental Protection Agency

Environmental Research Laboratory

Gulf Breeze, Florida 32561

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE, FL 32561

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Many chemicals enter the marine and estuarine environment through a variety of routes. These routes include dumping, direct application, outfalls, accidental spills, and land runoff or rainfall. Some of these compounds are toxic to the biota or may be converted to toxic products in nature. The fate and ecological effects of these chemicals in estuarine environments is part of the concern of the EPA Laboratory at Gulf Breeze, Florida. Toxicity results when an organism is exposed to a sufficient concentration of a compound. Therefore, fate greatly influences the cumulative effect of a chemical on the biota.

Toxicity effects can be attenuated by dilution of the pollutant below its toxic threshold or by physically removing it into a phase (sediments) where the chemical may not be available to indigenous organisms. Neither process alters the chemical structure of the toxicant. Chemical, photochemical and some biological processes bring about changes in chemical structure which may or may not alter the toxicity of the chemical or product. Whereas, microbial degradation can produce major changes in the chemical structure of the introduced chemical, mineralization is often the end result of bacterial and fungal activities.

The purposes of this paper are to: 1) provide selected examples in the marine and estuarine environments of known introductions of toxic chemicals, 2) describe how habitat differences affect biodegradation potentials in freshwater, estuarine, and marine environments; 3) and express some applications and concerns for the release of genetically-altered organisms into the environment.

CHEMICAL POLLUTANTS IN ESTUARINE AND MARINE ENVIRONMENTS

Historically, some organic and inorganic compounds have been considered serious environmental pollutants based on quantity produced, bioaccumulation,

toxicity, or adverse environmental effects. Although many of the problem chemicals e.g., chlorinated pesticides, are no longer produced or have been greatly reduced in use, some pose contemporary problems in our estuaries. Examples of pesticide residues accumulated by various marine organisms are shown in Table 1. Thus, while chlorinated hydrocarbon pesticides have been banned or restricted in use for years, they are still detected in marine biota. In some cases (California coast), the concentration appears to be higher than that found in other areas (Gulf of Mexico) years earlier (1). Kepone, a very persistent compound which accumulated in marine biota and sediments to significant levels, has caused a serious problem in the James River-Chesapeake Bay areas. Interestingly, results from field studies sometimes contradict laboratory studies; some monitoring studies have detected in marine biota compounds known to be degradable in the laboratory, e.g., pentachlorophenol (PCP) and parathion. These are examples of some of the kinds of problems that we are attempting to understand in our studies on microbial degradation of pollutants in the aquatic environments.

Other compounds which pose an environmental health hazard have accumulated in various estuarine-marine ecosystems (Table 2). Most sorb and accumulate in sediments. Recent evidence shows that polychlorinated biphenyls (PCB's) contained in the sediment are still bioavailable through the food chain and accumulate in estuarine organisms (4). Chemicals, such as those in Table 2, pose a number of problems for biodegradation or detoxification technology. They are almost always located in anaerobic sediments, and large variations in chemical structures require different biodegradation pathways (5). For example, chlorinated substitutions on aromatic hydrocarbons can prevent bond cleavage which normally occurs on the unsubstituted hydrocarbon. Usually another biochemical pathway or different enzyme is required to achieve

degradation. Additionally,they are almost always sequestered in sediments with compounds, such as heavy metals or pesticides, that may be toxic to the microbial population or at least inhibitory to the degradation of the other toxicants (6, 7). Some compounds (Table 2) found in the environment are known to be biodegradable, at least in laboratory studies; therefore, the enzymatic sequences exist in certain species, but are apparently not active within these environments. This may be due to a lack of capability, i.e., lack of enzymes or to a physical-chemical factor of the environment which prevents expression of the genetic capability. The evolution of plasmids or the addition of plasmids that confer the ability to withstand toxic concentrations of a variety of inorganic metal ions, Hg, Cd, and others (8, 9), or to express a greater degree of versatility in degradative pathways (10), could help to develop organisms that are tolerant of these toxicant combinations.

BIODEGRADATION IN AQUATIC ENVIRONMENTS

Laboratory studies have determined the biodegradability of chemical pollutants, such as hydrocarbons, chlorinated hydrocarbons, and various pesticides, including microbial biocides. Genetic studies have shown that the application of biotechnology can increase the detoxification capability of many organisms (11). Recent studies have demonstrated that the transfer of genetic information in nature is much more prevalent than previously perceived (12, 13). This versatility within the microbial community would suggest eventual biodegradation of any susceptible compound that enters the environment. However, we continue to find that biodegradation in the field differs from that predicted in laboratory studies.

A number of reasons for our inability to rely on laboratory-generated data to predict field results can be noted, and usually is related to laboratory

design (high nutrients, optimized oxygen concentrations, etc.) or to the nature of the microorganisms found in the field. A number of these conditions are listed in Table 3.

The environment in which an organism exists affects how it can function. Generally, organic compounds degrade more rapidly under aerobic conditions; therefore, oxygen level can affect the rate at which a compound degrades. Other environmental conditions include pH, temperature, and salinity. I will present information on the effect of salinity on biodegradation of xenobiotics. Low nutrient levels, both organic and inorganic, can retard biodegradation. An area requiring extensive study is the effect of sorption on biodegradation of organic compounds. Sediment, for example, sometimes stimulates degradation and other times may retard degradation (P.H. Pritchard, personal communication).

The chemical structure and concentration of the substrate are important considerations in biodegradation. Some chemical structures have never been demonstrated to be degradable by microorganisms even under the most optimal growth conditions, i.e., they are recalcitrant. Polyaromatic hydrocarbons above three rings undergo some transformation but no significant degradation has been demonstrated. Microbial degradation at low concentrations of toxic organic chemicals may utilize different degradative pathways than occur with the same compounds at high concentrations (M. Alexander, personal communication). Combinations of organic chemicals or chemicals and metals can affect the degradation of certain pollutants; evidence will be presented later to demonstrate such an affect. This may be an area of application of genetic engineering to develop organisms that are resistant to heavy metal contamination but retain the ability to degrade organic contaminants in the environment. The third major consideration of factors controlling biodegradation in the environment is the microbial population. The

organisms must possess the genetic capability to degrade the pollutant, including transport of the substrate across the membrane. The number of bacteria in a system has a direct relationship with the rate of degradation of the pollutant, especially the number of the specific degraders in a given environment. Products of catabolism can inhibit the degradation process, resulting in intermediate accumulation (5). Predation of the microbial population, although considered important in biodegradation of organic compounds, has not been fully investigated. These conditions all affect how a compound will or will not be degraded in an aquatic environment. Our goal is to determine how such conditions, individually or collectively, affect the degradation process.

Kaplan (14) pointed out that in order to extrapolate laboratory data to the field, we must reorient our approach to understanding quantitatively those environmental factors that control biodegradation in the environment. This approach considers the microorganism as the driving mechanism, but identifies the environment and substrate as the controlling and limiting factors. For example, Di-2-ethylhexyl phthalate (DEHP), when it occurs as a plasticizer in polyvinyl chloride (PVC) films, is resistant to microbial degradation, but DEHP alone is susceptible to microbial attack in the laboratory, and can even be mineralized (15). Giam (16) has shown that DEHP is present in the open ocean to the extent that he considers phthalate esters to be a class of marine pollutants. Generally, higher concentrations of DEHP are found in water, sediment, and air in coastal regions than in the open Gulf of Mexico. If these compounds can be metabolized, why do they accumulate in the marine environment, not only in anaerobic areas, but also in habitats where one can assume dissolved oxygen to be present?

These and other cases have led my laboratory to probe the differences in

biodegradation potential between estuarine and freshwater habitats, with the overall goal of characterizing the environment and understanding the role of the microbial community in the biodegradation process. Several case studies on selected synthetic organic chemicals suggest that degradative processes in estuarine and marine environments differ fundamentally from those in terrestrial and freshwater environments.

Early studies in our laboratory on nitrilotriacetic acid (NTA), a proposed phosphate substitute for detergent mixtures, showed that NTA is not degraded by estuarine microorganisms found in the water column (17). Other studies, however, showed that NTA is biodegradable in river water (18), sewage treatment plants (19), and soils (20). We found no evidence of NTA degradation in the saline environments. Whether the NTA-degrading bacteria (entering from the freshwater river) failed to survive in the estuarine areas, or if the enzymes of surviving microbes were not functional, was not determined. For certain freshwater microbes, the latter phenomenon has been observed (J.M. Tiedje, Michigan State University, personal communication). Interestingly, NTA-degrading bacteria have been observed in ocean environments (3.5% salinity) (Wm. Gledhill, Monsanto Co., personal communication). These later studies confirmed the lack of NTA-degradation in estuarine environments, but showed the capability in higher, constant salinity environments. No explanations have been given for these differences within saline environments.

Other investigators (21) have demonstrated that organic compounds (glucose and acetate), which usually degrade rapidly in freshwater environments, become almost refractory in saline wastewaters. These studies showed a 50% decrease in utilization of organic carbon, as measured by BOD, when salt concentration was increased from 0% to 3%. No evidence was presented for the cause of the reduced degradation, but it appears that the salinity affected the enzymatic

activity of the microbial populations. Ward and Brock (22) extended this evidence to hypersaline environments (< 3%) in a study that strongly suggests occurrence of a general reduction of metabolic rate at extreme salinities and raises doubt about biodegradation of simple hydrocarbons in hypersaline environments. Investigations into the activity and population densities in various coastal environments by Wright (23) have shown decreased specific activities and population densities as water depth and salinity increase. However, he postulates an adaptive strategy for the natural heterotrophic bacteria that involves reversible changes from functional dormancy to a high degree of activity. Therefore, it is possible that populations in estuaries can adjust to faster metabolic activity if the proper conditions are present when pollutant enrichment occurs.

Experimental Studies with Eco-core

To determine how estuarine microbial communities respond after introduction of a xenobiotic, we devised experiments in a laboratory test system called "eco-core" (24). Early in studies using our system, we examined the effect that one toxic chemical may have on the degradation of another known, biodegradable toxicant. Eco-cores from Kepone-contaminated sediments of the James River (Virginia) were used to determine the effect of Kepone on the biodegradation of methyl parathion. Additional cores were taken from an uncontaminated area in a Florida saltmarsh (Range Point) and treated with Kepone prior to testing for methyl parathion degradation. These studies showed that Kepone is inhibitory to biodegradation processes that normally occur (Figure 1). Results indicate that inhibition is proportional to Kepone sediment concentration. These results indicate serious impairment of a degradation process by another pollutant and emphasize the effects of

environmental conditions and other contaminants on the degradation potential of indigenous microorganisms.

Other eco-core studies examined the degradation rates of the "natural" microbial community when allowed to "adapt" after exposure to a xenobiotic. Studies were performed with water-sediment systems (eco-cores) taken from a salt marsh and riverine sources. Systems were tested for biodegradation of the model compounds methyl parathion (MP) and p-nitrophenol (PNP) (25). $^{14}CO_2$ released from radioactive parent compounds was used as a measure of mineralization. River bacterial communities preexposed to PNP at concentrations as low as 45 µM degraded the nitrophenol much faster than did control communities (Figure 2). Similar results were obtained with MP; river bacterial communities adapted to faster degradation, although higher substrate concentrations were required. Saltmarsh bacterial communities did not adapt to degrade PNP or MP (Figure 3). Para-nitrophenol-degrading bacteria were isolated from freshwater river samples, but could not be detected in saltmarsh samples. These studies suggest that specific biodegradation capabilities may be present in one aquatic environment, but not in another aquatic environment. The exact cause of these differences in biodegrdation rates was not determined; however, there appeared to be an absence of microorganisms in the saltwater system capable of degrading PNP although PNP-degrading organisms were carried by freshwater into the estuary (25).

Additional estuarine and marine sites (6 sites) and freshwater sites (5 sites) were sampled to determine if the previous results were truly representative of the subject environments. All freshwater sampling sites adapted to a faster rate of degradation after exposure to PNP. When the experiment was repeated with estuarine and marine samples, there was no adaptation to faster biodegradation (26).

To determine if the microbial response phenomenon was compound-dependent, trifluralin, PNP, p-cresol, and 2,4-dichlorophenyoxyacetic acid (2,4-D) were tested in eco-cores with water and sediment samples from the river. Figure 4 shows the disappearance of parent compound and the evolution of $^{14}CO_2$ from control and pre-exposed cores. The systems adapted to a faster degradation rate for PNP and 2,4-D, but not for p-cresol or trifluralin (26).

Such studies clearly indicate the differences in microbial communities of two nearby aquatic systems. However, no explanation was established as to the nature or cause of the differences. Although no PNP-degrading microorganisms were isolated from the estuarine system, this does not mean the genetic information or the potential was not present. It may not have been expressed or it may have been inhibited by some environmental parameter. Although it is assumed that organisms carrying the genetic information entered the estuarine environment because they were isolated in the freshwater flowing into the estuary, the genetic information either was not expressed or lost. No specific genus was indicated in these studies although G(-) rods were the morphological-type cells isolated as PNP-degraders.

Investigations, using four test chemicals, para-chlorophenol (4-CP), para-nitrophenol (PNP), methyl parathion (MP), and pentachlorophenol (PCP) in 3 different saline environments and a river, were conducted to empirically determine differences in degradation rates and mineralization rates in these distinctly different but adjacent aquatic environments. Radiolabelled compounds were added to eco-cores filled with samples from the four sites. Degradation rates were measured by collecting the $^{14}CO_2$ that evolved, then extracting and quantitating the parent compound, metabolites, and unextractable C-14.

PNP degradation was fastest in the river system, followed by the

saltmarsh, bay, and Gulf systems (Figure 5). 4-CP was degraded quite rapidly in all systems, but more slowly in the saltmarsh system. Interestingly, 4-CP was mineralized more extensively as the salinity increased and biomass decreased (Figure 6) with 100% of the theoretical ^{14}C being evolved as $^{14}CO_2$ in the Gulf systems. PCP was not mineralized to any significant extent in any system, although some $^{14}CO_2$ was evolved in the river and Gulf systems. The parent compound was degraded extensively in the river and saltmarsh systems (Figure 7), but only limited degradation occurred in the higher salinity environments. Methyl parathion hydrolyzes in aquatic environments to PNP, which is subsequently degraded (24). The parent compound, MP, hydrolyzed at essentially the same rate in all systems and the evolution of $^{14}CO_2$ was the same as for PNP (Figure 5).

Correlations of heterotrophic activity with biomass data do not fully explain the results of these studies. Biomass decreased with increasing salinities, but in some cases, i,e., mineralization of 4-CP in the Gulf system, activity was much greater. In the case of MP and PNP, the decrease in biodegradation rate correlates with the decrease in biomass. However, even here mineralization is not proportional to the biomass or activities of the environment. These results indicate varied degradative responses to organic substrates by microbial communities from different environments probably result from differences in growth limiting conditions at each environmental site.

The examples cited demonstrate the variable response of microbial populations in adjacent aquatic environments. It would seem highly probable that microbes existing in one environment (river) would be transported into the other (bay or saltmarsh). Their existence and proliferation would be dependent upon nutrient content, pH, temperatures, oxygen level, and environmental and

other factors within the receiving environment (27).

In addition, it seems highly probable that certain enzyme functions expressed in one environment could be inhibited in another because of salt-induced conformational changes in the enzyme. Before we can predict if a particular organism can survive and function in a new environment, we need to understand more about how environmental factors affect the functions of a microbial population.

CONCERNS FOR RELEASE OF GENETICALLY ALTERED ORGANISMS

Any discussion of the application of genetically altered organisms should include the conditions and circumstances which may affect the survival and perpetuation of a genetically altered species in the environment and the potential hazards involved with its release. For purposes of this discussion, survival differs from perpetuation in that an organism or trait could survive in an environment for a duration of time but not perpetuate itself through multiplication and thus would eventually be "diluted" out of the ecosystem. Table 4 condenses and summarizes concerns for the survival and potential hazards of genetically altered microorganisms as discussed by Curtiss (28).

Survival and Perpetuation. In order to achieve, functionally, the purpose for which an organism was developed for release into the environment, it almost always must be able to survive and perpetuate within a given environment. As noted earlier, the characteristics of the receiving environment will affect whether and how the organism can exist and function. Even in natural systems, certain traits or capabilities can be lost when environmental conditions change. The lack of the NTA-degrading potential of bacteria within a freshwater river flowing into an estuary is an example. The nature of the organism itself must be considered. A totally laboratory-attenuated host may not survive under conditions in a natural environment. On the other hand, the

additional DNA could alter the environment to allow survival of the host. The ability of genetically engineered organisms or the trait to reproduce is a critical factor for the perpetuation of the population or characteristic. If the organism is unable to reproduce, the population will die out. The survival and perpetuation of the host necessitates that the foreign DNA confer some selective advantage or at least no disadvantage to the genetically engineered organism.

Survival of the genetically altered trait can be affected by survival of the host cell or the vector involved in the genetic transfer. If the host doesn't survive, the trait will more than likely die with it. However, the trait's survival could be increased if the foreign DNA or altered DNA fragment is passed to another organism in the environment. The potential for trait survival seems highly probable with certain genetically altered species carrying promiscuous plasmids capable of infecting a broad spectrum of microorganisms (i.e., gram negative bacteria). The survival and perpetuation of the DNA and new trait could be facilitated by transfer to another vector or host in the environment which would be better adapted to growth within a particular ecological niche.

Some information on survival and trait transfer can be developed from methods using simulated environments (microcosms), such as eco-core. It will not be practicable to simulate all of the environmental conditions or the combinations of environmental conditions that might allow reproduction, growth, and survival of genetically altered organisms. But these methods have been used to simulate environmental conditions in the laboratory on a site-specific basis.

Transmissibility. The exchange of genetic material between species and genera

of bacteria is so common that it is highly likely that transfer of altered DNA will occur if the host survives and that transfer will more than likely occur via transduction or conjugational plasmid transfer (28). However, with sufficient information on the nature of the host cell, transmissibility in the laboratory, and the nature of the receiving environment, we may be able to evaluate this potential. Laboratory studies can evaluate the potential for transfer of genetic material from one microbe to another. However, those studies will provide data on only the potential for genetic exchange among the species tested and under the conditions employed in the laboratory. The likelihood for exchange with other species in the environment remains unknown and requires investigation. It is not practical to test all possible situations with some altered organism; it may be possible to anticipate probable transfer through laboratory testing.

CONCLUSIONS

Many factors control and regulate biodegradation in aquatic environments. Some of these factors have been discussed in this paper. In addition to the more obvious constituents of an environment necessary for the organism's growth, certain physical/chemical factors may inhibit or alter the function within a particular ecosystem. One of the more obvious discussed here was the loss of certain biodegradation functions in the transfer from freshwater to a saline environment. Another factor was the inhibitory effect of one pollutant on the biodegradation of another. Biodegradation potentials in different saline environments for xenobiotics of varied chemical structures were dramatic when compared with freshwater, saltmarsh, estuarine and marine environments. Some of the reasons regarding how each factor affects the biodegradation functions were discussed briefly. Certainly a lot of work remains to be done to understand the biodegradation function in a particular environment.

The release of genetically altered microorganisms in the environment creates a whole new set of questions which relate to the investigations just presented. It is, of course, necessary to know how and why a particular function exists in one environment and not in another in order to achieve the results desired from releasing genetically altered organisms. If it is known that a particular trait can function in a particular environment, then it is possible to genetically manipulate an organism that contains the desired traits. Release of that organism creates another set of questions related to interference and destruction of natural ecosystem functions. The questions of survival of the host (or altered organism), transmissibility of the trait, or ecosystem disruption can be answered somewhat through studies similar to those presented here. These studies using laboratory microcosms and the problems of control of biodegradation in the environment, as well as the limitations and applications of genetic engineering, are inter-related. Advancements in any area will contribute to advancement in the other areas.

Figure 1. Effects of Kepone on Methyl Parathion (MP) mineralization in saltwater eco-cores. Range Point (Florida saltmarsh) eco-cores sediments were supplemented immediately with 500 ppm Kepone and the water treated with 200 ppb ^{14}C-MP. James River cores were constructed with Kepone-contaminated sediments (high = 600 ppm Kepone, low = 140 ppm Kepone) from sites in the James River Estuary and Range Point water which was treated with 200 ppb ^{14}C-MP. $^{14}CO_2$ was trapped and measured as radioactivity. Source: Proceed. 12th Ann. Conf. on Environ. Toxicol. 1981. p. 354.

Figure 2. Mineralization in preexposed Escambia River cores. Eco-cores were taken at the Escambia River site and spiked immediately with unlabeled MP or PNP at concentrations of 0.45 and 180 µM. Control cores received no supplement. Cores were incubated 100 h, water was removed and replaced with filter-sterilized river water, and cores were respiked with (^{14}C)MP or (^{14}C)PNP at a concentration of 180 µM (A) or 0.45 µM (B). $^{14}CO_2$ release was followed during subsequent incubation. Source: Appl. Environ. Microbiol. 40:726-734.

Figure 3. Mineralization in preexposed Range Point eco-cores. Cores were prepared at the Range Point sampling site and spiked immediately with unlabeled MP or PNP at concentrations of 180 µM (A) or 0.45 µM (B). Control cores received no supplement. Cores were incubated 250 h, water was removed and replaced with filtered Range Point water, preexposed and control cores were then respiked with (^{14}C) MP or (^{14}C)PNP at concentrations of 180 µm (A) or 0.45 µM (B), and $^{14}CO_2$ release was followed during subsequent incubation. Note the x-axis scale relative to that in Fig. 3. Source: Appl. Environ. Microbiol. 40:726-734.

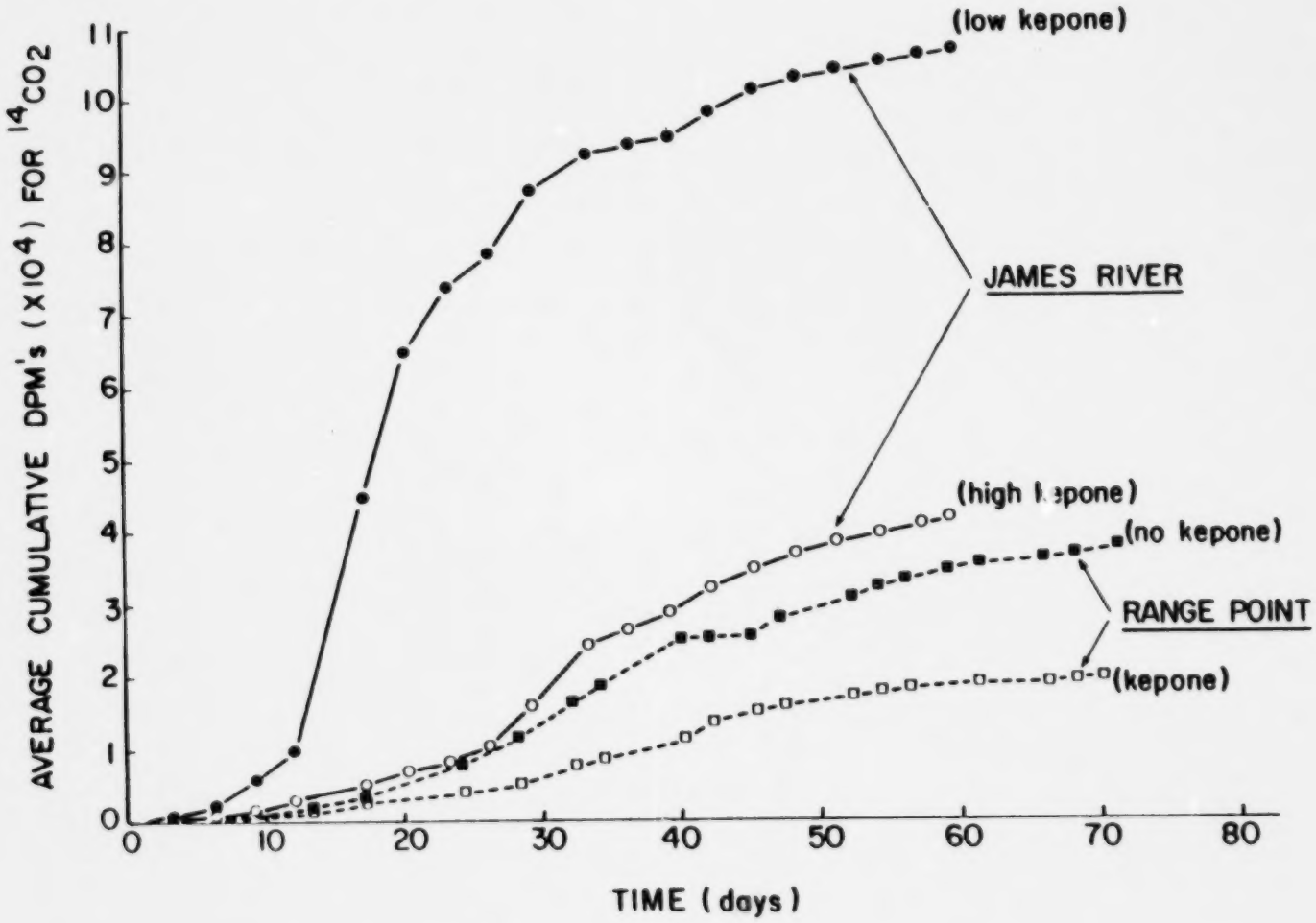
Figure 4. Mineralization of radiolabeled test compounds by preexposed and control populations from the Escambia River. (A)PNP, (B) 2,4-D, (C) p-Cresol, (D) Trifluralin. Eco-cores from the Escambia River test site were exposed to the test compounds at concentrations of 1.08 µM; control cores received no supplement. After 8 days of incubation at 20°C, all cores were tested with 1.08 µM radiolabeled compound. During subsequent incubation, $^{14}CO_2$ release was measured, and water samples were analyzed for parent compounds by high-pressure liquid chromatography or gas chromatography. The values shown are averages of data from duplicate cores; sample-to-sample variation never exceeded 10% of the mean. Source: Appl. Environ. Microbiol. 45:428-435.

Figure 5. Mineralization and loss of radiolabeled p-nitrophenol (PNP) from Ecocores taken from Escambia River (10°/oo salinity), Range Pt. saltmarsh (15°/oo), Escambia Bay (20°/oo) and near off-shore Gulf of Mexico (35°/oo). Cores were taken in various environments, treated with 200 ppm ^{14}C-PNP and incubated 21 days at 25°C. During incubation, $^{14}CO_2$ release was measured daily and a core was sacrificed every 7 days and parent compound analyzed by gas chromatography. Sterile cores were treated with 2% formalin and analyzed as above.

Figure 6. Mineralization and loss of radiolabeled 4-chlorophenol (4-CP) from Ecocores taken from Escambia River (10°/oo salinity), Range Pt. saltmarsh (15°/oo), Escambia Bay (20°/oo) and near off-shore Gulf of Mexico (35°/oo). Cores were taken in various environments, treated with 200 ppm

^{14}C-4-CP and incubated 21 days at 25°C. During incubation, $^{14}CO_2$ release was measured daily and a core was sacrificed every 7 days and parent compound analyzed by gas chromatography. Sterile cores were treated with 2% formalin and analyzed as above.

Figure 7. Mineralization and loss of radiolabeled pentachlorophenol (PCP) from Eco-cores taken from Escambia River (10^{o}/oo salinity), Range Point saltmarsh (15^{o}/oo), Escambia Bay (20^{o}/oo) and near off-shore Gulf of Mexico (35^{o}/oo). Cores were taken in various environments, treated with 200 ppm ^{14}C-PCP and incubated 21 days at 25°C. During incubation, $^{14}CO_2$ release was measured daily and a core was sacrificed every 7 days and parent compound analyzed by gas chromatography. Sterile cores were treated with 2% formalin and analyzed as above.


AVERAGE CUMULATIVE DPM's (X10⁴) FOR ¹⁴CO2
0
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2
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4
5
6
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8
9
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TIME (days)
(low kepone)
JAMES RIVER
(high kepone)
(no kepone)
RANGE POINT
(kepone)

Figure 1

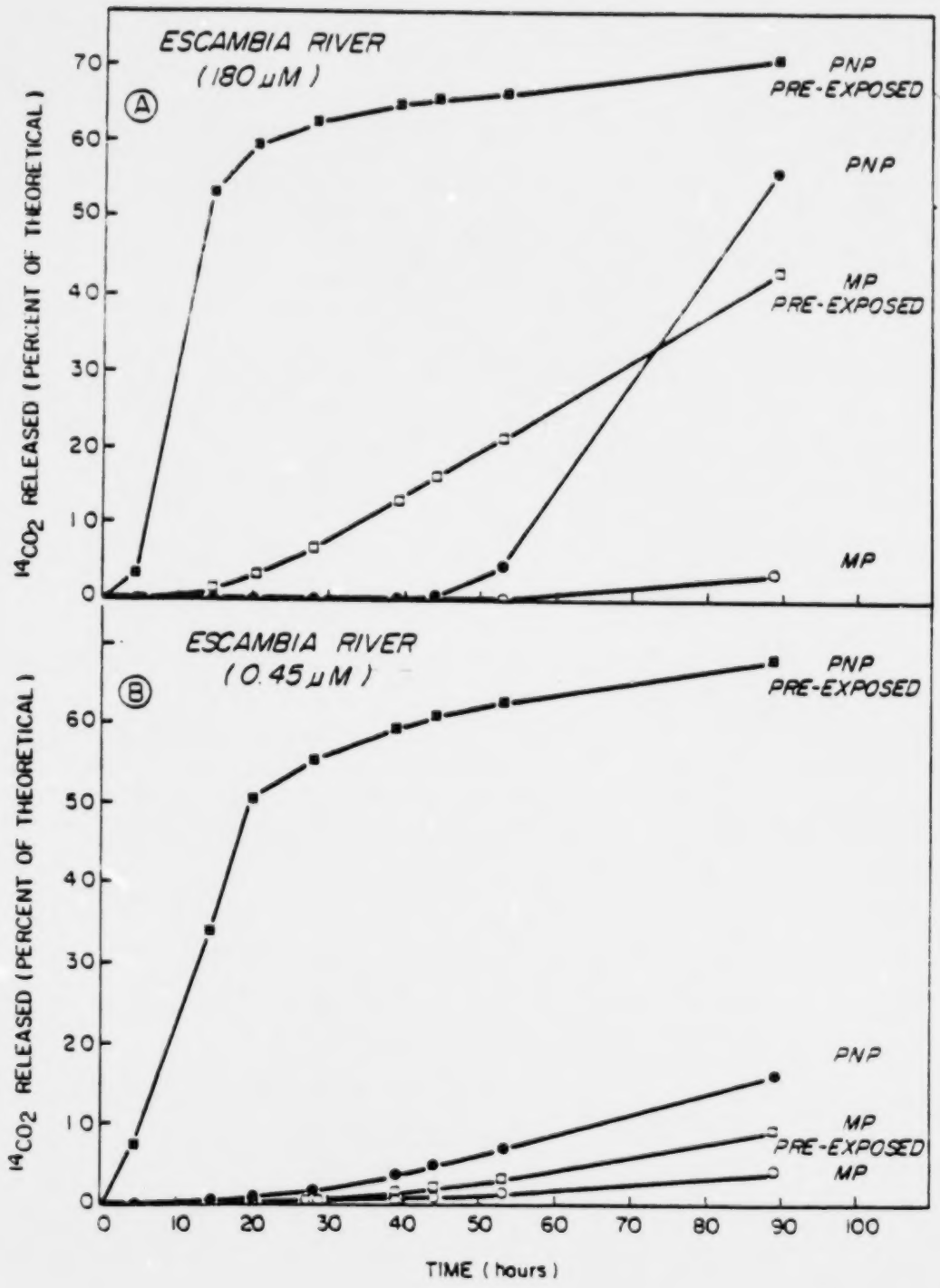
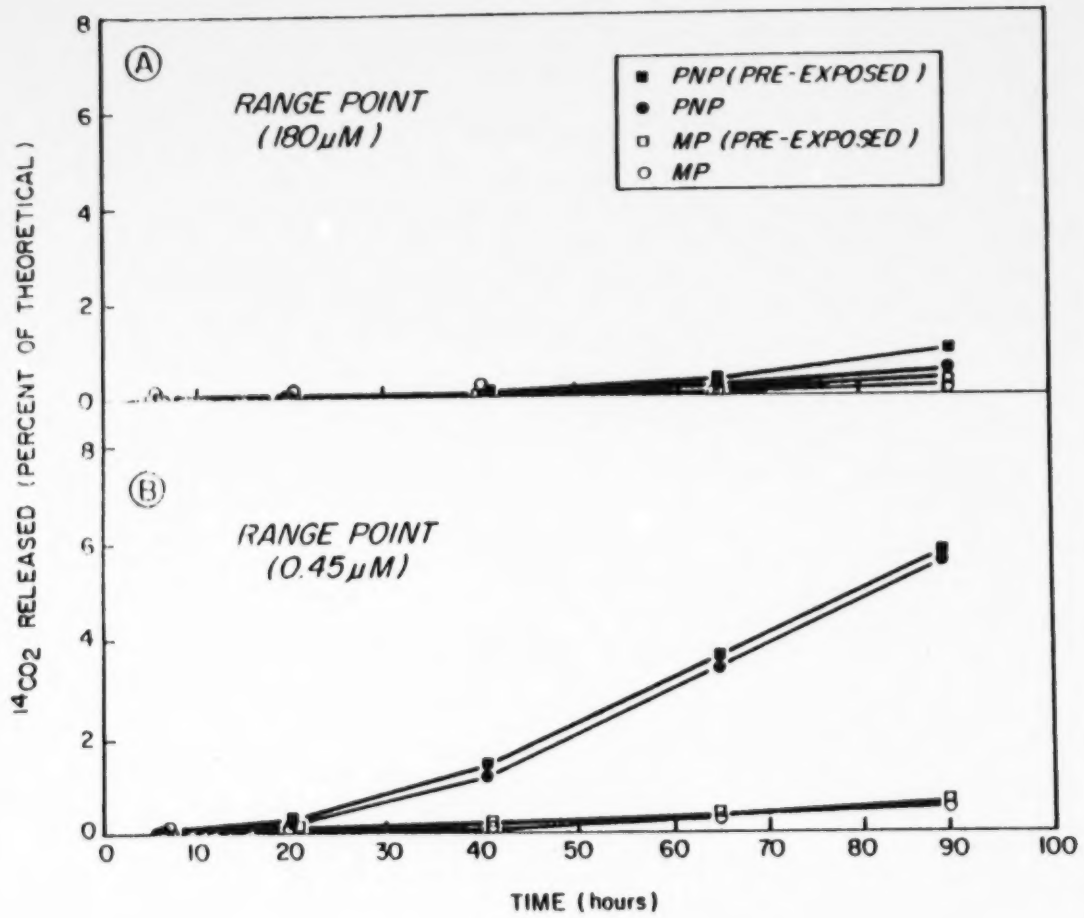

ESCAMBIA RIVER
(180 µM)
A
PNP
PRE-EXPOSED
PNP
MP
PRE-EXPOSED
MP
$^{14}CO_2$ RELEASED (PERCENT OF THEORETICAL)
ESCAMBIA RIVER
(0.45 µM)
B
PNP
PRE-EXPOSED
PNP
MP
PRE-EXPOSED
MP
$^{14}CO_2$ RELEASED (PERCENT OF THEORETICAL)
0 10 20 30 40 50 60 70 80 90 100
TIME (hours)

Figure 2


(A)
RANGE POINT
(180μM)
PNP (PRE-EXPOSED)
PNP
MP (PRE-EXPOSED)
MP
(B)
RANGE POINT
(0.45μM)
$^{14}CO_2$ RELEASED (PERCENT OF THEORETICAL)
TIME (hours)
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80
90
100

Figure 3

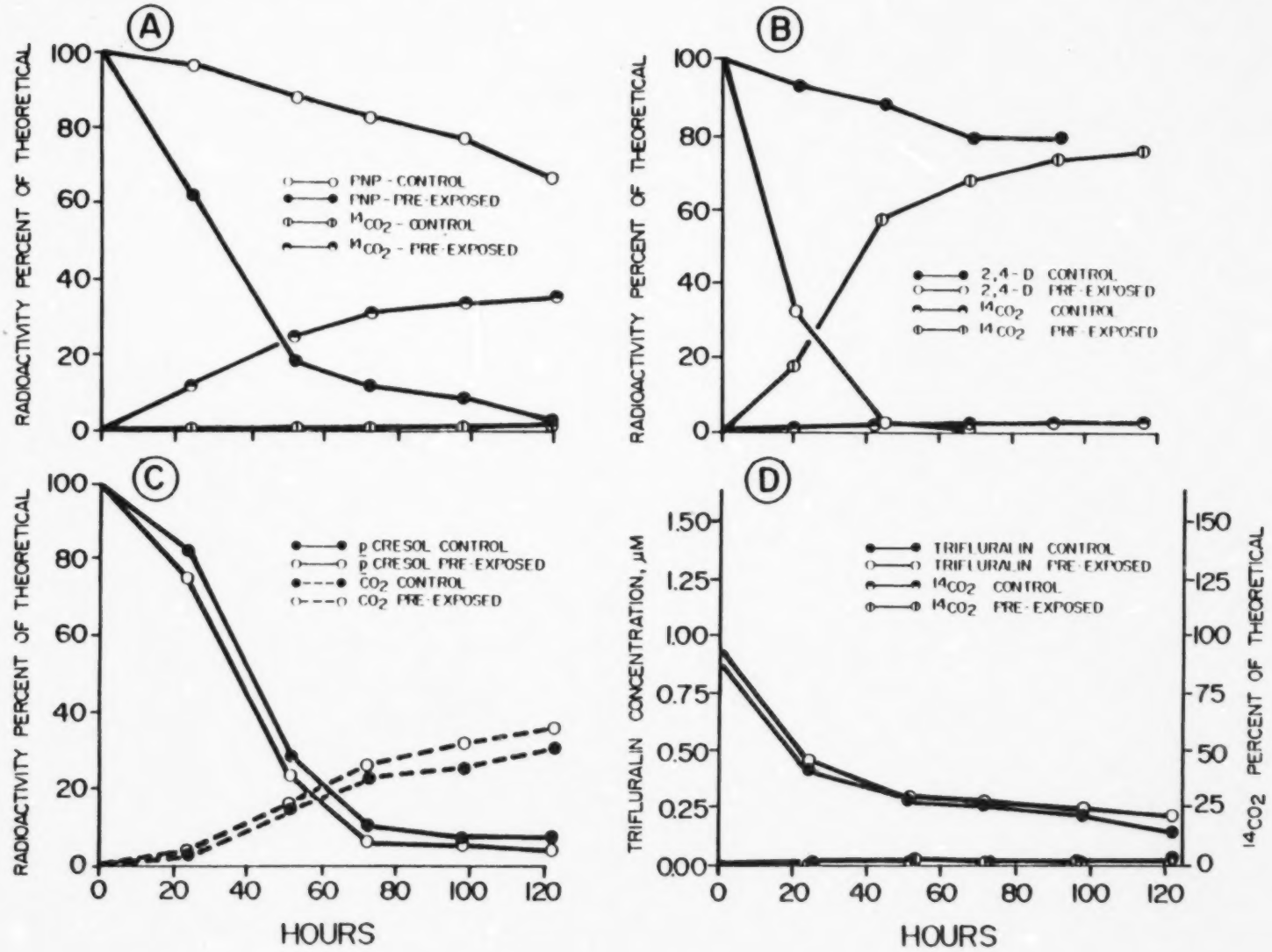
A
RADIOACTIVITY PERCENT OF THEORETICAL
PNP - CONTROL
PNP - PRE-EXPOSED
$^{14}CO_2$ - CONTROL
$^{14}CO_2$ - PRE-EXPOSED
B
RADIOACTIVITY PERCENT OF THEORETICAL
2,4-D CONTROL
2,4-D PRE-EXPOSED
$^{14}CO_2$ CONTROL
$^{14}CO_2$ PRE-EXPOSED
C
RADIOACTIVITY PERCENT OF THEORETICAL
p CRESOL CONTROL
p CRESOL PRE-EXPOSED
CO_2 CONTROL
CO_2 PRE-EXPOSED
HOURS
D
TRIFLURALIN CONCENTRATION, µM
$^{14}CO_2$ PERCENT OF THEORETICAL
TRIFLURALIN CONTROL
TRIFLURALIN PRE-EXPOSED
$^{14}CO_2$ CONTROL
$^{14}CO_2$ PRE-EXPOSED
HOURS

Figure 4

FATE OF PARANITROPHENOL IN ECO-CORES FROM FOUR DIFFERENT ENVIRONMENTS

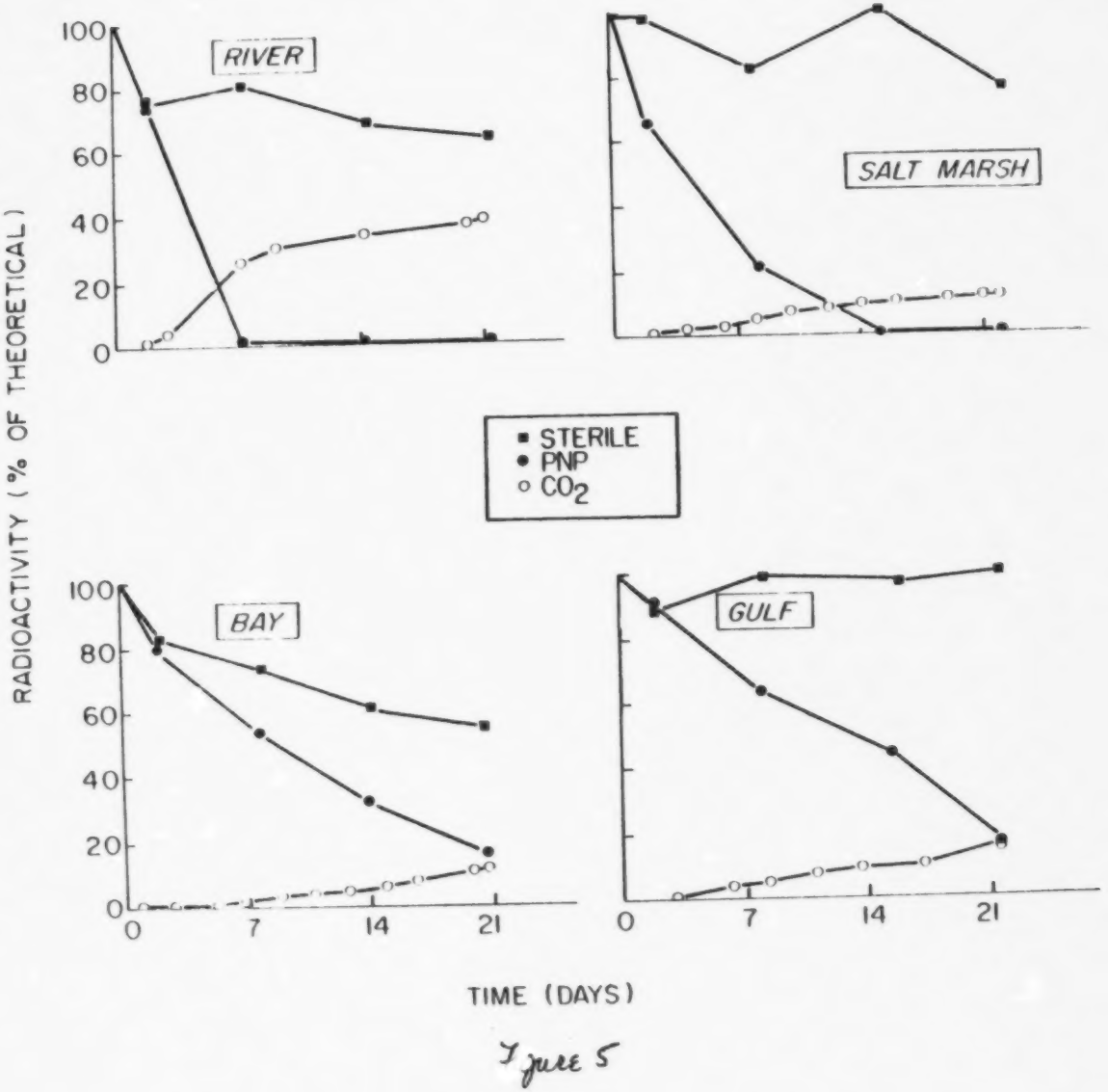

Figure 5

FATE OF PARACHLOROPHENOL IN ECO-CORES FROM FOUR DIFFERENT ENVIRONMENTS

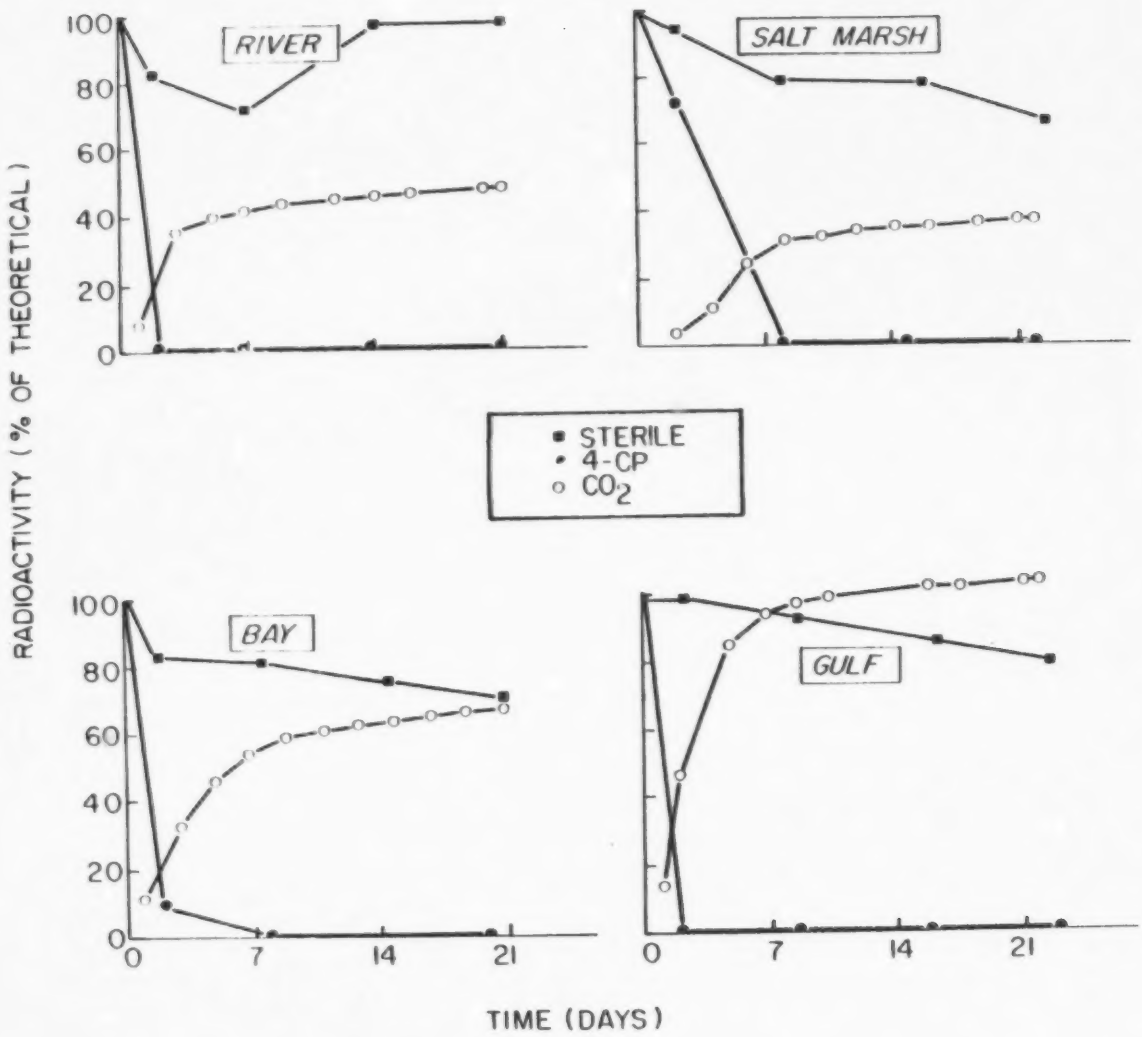

Figure 6

FATE OF PENTACHLOROPHENOL IN ECO-CORES
FROM FOUR DIFFERENT ENVIRONMENTS

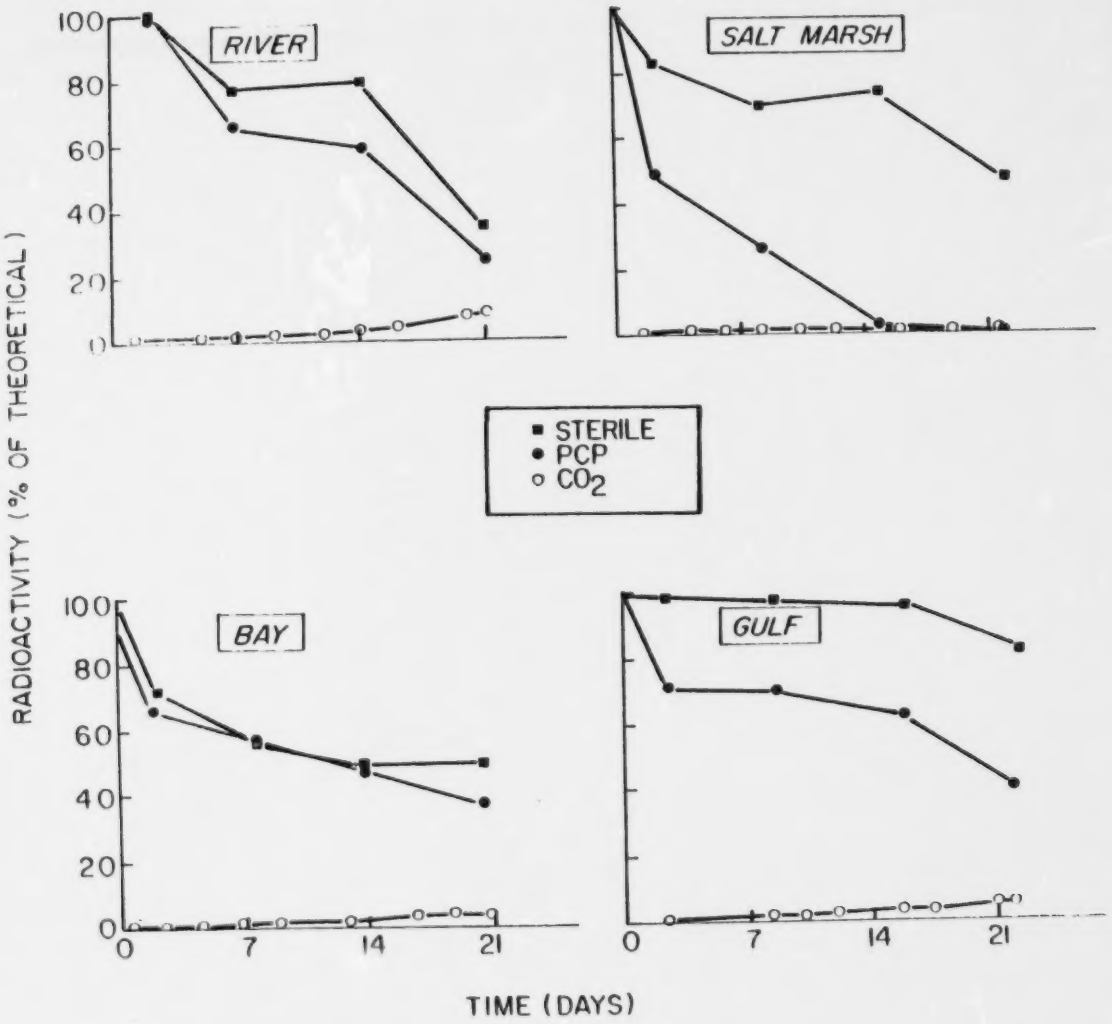

Figure 7

Table 1. Pesticide residues in various marine biota

Compound	Species	Location	
DDT	Striped Bass	Gulf of Mexico	0.63 µg/g
	Fish (various)	Gulf of Mexico	0.1 - 6.0 µg/kg
	Spanish Mackerel	Gulf of Mexico	0.008 - 0.319 µg/ℓ
	Common Dolphin	California Coast	13 - 36 mg/ℓ
Kepone	Blue Crab	James River	0.01 - 0.55 µg/g
	Blue Crab	Chesapeake Bay	0.634 - 1.36 µg/g
PCP	Oyster	Galveston Bay	3.40 - 8.30 ng/g
Parathion	Clam	Massachusetts	0.02 - 0.03 mg/g

References 1, 2, 3.

Table 2. Pollutants found in estuarine/marine environments*

Compound	Location	Concentration**
Polychlorinated Biphenyls	Hudson River	0.15 - 1.59 µg/g
Brominated Organics	Sewage Treatment Plant Effluents	0.1 - 50 µg/ℓ
Hydrocarbons		
Naphthalenes	--	0.2 - 496 µg/kg
Polycyclic		
Benzo-pyrene	Charles River	8000 µg/kg
Anthracene Gp.	Mobile Bay	2 - 50 µg/kg
Acenaphthene Gp.	--	320 - 100 µg/ℓ
Heavy Metals		
Mercury	New England	305 - 568 µg/g
Cadmium	New York	1.9 - 119.7 µg/g
Lead	Mass.	0 - 8.56 µg/g

* Ref. 1, 2, 3, 4.

** kg or g = sediments; ℓ = water

Table 3. Conditions affecting the environmental biodegradation of organic toxicants

1. Environmental conditions

   - Anaerobiosis (Eh)
   - pH, Temperature, (Salinity)
   - Nutrient Levels (Organic and Inorganic)
   - Sorption to Particulates

2. Chemical parameters

   - Structure
   - Concentration
   - Toxic Chemicals (Mixtures)
     Metals, Organics

3. Microbial parameters

   - Genetic Capabilities (Including Transport)
   - Size of Population
   - Microbial Products
   - Predation/Parasitism

Table 4. Survival and perpetuation of genetically altered organisms or traits

A. Nature of microbial host and cloning vectors

1. Host survival depends on suitable environment for multiplication
2. Trait survival by transfer of foreign DNA to environmental recipient

B. Ecological niche of altered organism and original

1. Natural ecological niche of host organism
2. Contribution of foreign DNA to alter ecological niche
3. Distance between occupiable niche and laboratory or containment facility

C. Transmissibility

1. Foreign DNA fragments frequently transferred in nature or in laboratory
   - Transduction via phage
   - Plasmid transfer via conjugation
2. Recipient of foreign DNA in nature would have higher probability of survival

D. Contributation of foreign DNA

1. Offers selective advantage (or no disadvantage) to survival of host of vector
2. Most cases, cloned DNA will be detrimental to competitive survival of cells or vectors

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